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Activation of Variants of Hypoxanthine-Guanine Phosphoribosyl Transferase by the Normal Enzyme

(isoenzymes/Lesch-Nyhan syndrome/hyperuricemia/electrophoresis)

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ABSTRACT **Deficient hypoxanthine-guanine phosphoribosyl transferase (HGPRT; EC 2.4.2.8) enzymes from erythrocytes of patients with hyperuricemia and with the Lesch-Nyhan syndrome migrate 15% faster in polyacrylamide gel disc electrophoresis than the normal enzyme. A half-sister of two males with partial deficiency, who had 34% of normal HGPRT activity in her erythrocytes, yielded profiles containing two distinct zones of activity; one corresponded to the enzyme found in normal individuals and one to the variant of her half-brothers. However, in her profile her variant enzyme showed notably greater activity than that observed in her half-brothers. This increase was due to an activation of the variant by normal enzyme. Electrophoresis of mixtures of normal enzyme with partially deficient enzymes from patients with hyperuricemia and with the Lesch-Nyhan syndrome also led to activation of deficient HGPRT variants by normal enzymes. Deficient variants were also activated by normal enzyme on filtration through Sephadex G-25. Experiments in which deficient variant enzymes were activated with purified normal enzyme labeled with ^{125}I indicated that deficient enzymes incorporate components of the normal enzyme. No such activation of deficient enzymes was ever obtained when mixtures of deficient and normal enzymes were put together in a test tube.**

The enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT; EC 2.4.2.8) catalyzes transfer of the 5-phosphorylribose moiety of 5-phosphorylribose-1-pyrophosphate (PRPP) to hypoxanthine and guanine to form inosine monophosphate and guanosine monophosphate. A virtually total deficiency of this enzyme activity is characteristic of the Lesch–Nyhan syndrome (1, 2), in which hyperuricemia accompanies cerebral dysfunction and self-mutilative behavior. Patients with a partial deficiency of this enzyme have hyperuricemia, and they may have renal stone disease or gout, but they do not have central nervous system abnormalities (3, 4). Thus, patients with a partially deficient enzyme are distinct phenotypically from those with the Lesch–Nyhan syndrome (5).

The enzyme is coded by a gene on the X-chromosome (3, 6). Heterozygous females who are carriers of the gene for total deficiency of HGPRT have two distinct cell populations, one in which the enzyme is normal and the other in which the enzyme is deficient (7, 8). However, in the hematopoietic system of these same heterozygotes, the expression of this gene is hemizygous and their erythrocytes contain only the normal enzyme (9). In contrast, in heterozygous females who are carriers of the gene for partial deficiency of HGPRT, the erythrocytes may contain both the normal and the deficient enzyme (10, 11).

With a highly sensitive method of polyacrylamide gel electrophoresis, (12, 13) it has been possible to demonstrate different molecular forms of HGPRT in erythrocytes of normal individuals, patients with the Lesch–Nyhan syndrome, and those with partial deficiency (11). During electrophoretic studies of HGPRT, we have observed a presumably heterozygous female with large zones of activity in the areas of both the normal and the partially deficient variants. This appeared to represent an activation of the deficient variant. In confirmation of this hypothesis, electrophoresis of a mixture of hemolysates from patients with the partially deficient enzyme with hemolysates from individuals with normal enzyme yielded similar profiles, with large areas of activity in the zone of the deficient enzyme (11). A systematic examination of the activation of variant enzymes by normal enzyme was undertaken in order to elucidate the nature of the reaction.

MATERIALS AND METHODS

The isoenzymes of HGPRT were separated by polyacrylamide gel disc electrophoresis (12, 13). HGPRT activity was assayed by a method in which the nucleotide product of the reaction is precipitated with lanthanum chloride (14). In some experiments gels were incubated in solutions containing [8-^{14}C]hypoxanthine and [8-^{14}C]adenine substrates, which permitted simultaneous detection of HGPRT and adenine phosphoribosyl transferase (12). The zone of adenine phosphoribosyl transferase was used as an internal reference for the size of the sample and the electrophoretic separation. Quantitation of enzyme activity on the gel has been described (13). The reproducibility of the method approximates 3% in data that will be published elsewhere. The isolated normal HGPRT enzyme has been purified about 8000-fold (Bakay, B., Svenson, P., and Nyhan, W. L., unpublished data). It was labeled with ^{125}I in the presence of chloramine-T (15) to a specific activity of 5.5×10^5 dpm/mg of protein.

RESULTS

Electrophoretic profiles of normal and variant enzymes

Radioelectropherograms produced by hemolysates from individuals with normal HGPRT-M enzyme differ qualita-

Abbreviation: HGPRT, hypoxanthine-guanine phosphoribosyl transferase.

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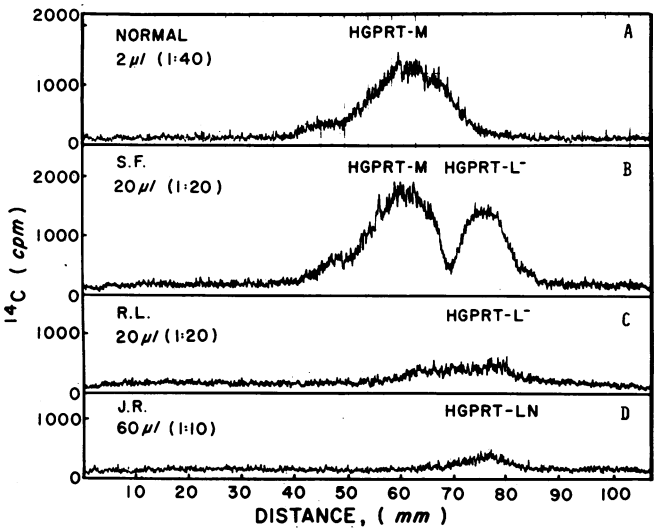

FIG. 1. Radioelectropherograms produced by hemolysates from (*A*) an individual with normal activity; (*B*) S. F., a heterozygote carrier of partial deficiency; (*C*) R. L., a patient with partial deficiency (4% of normal activity); and (*D*) J. R., a patient with the Lesch–Nyhan syndrome. Gels (*A*), (*B*), and (*C*) were incubated in substrate solutions containing 5 nmol (0.25 μCi) of [8-^{14}C]hypoxanthine per ml, and gel (*D*) in substrate containing 10 nmol (0.5 μCi)/ml; they were fixed in $LaCl_3$ solution, washed in water, and screened for ^{14}C.

tively, as well as quantitatively, from those of hyperuricemic males with the partially deficient HGPRT-L$^-$ variant and of patients with the Lesch–Nyhan syndrome (Fig. 1). Hemolysates containing the normal enzyme yielded a broad zone of radioactivity between 40 and 70 mm along the gel. There was a smaller and a larger component, which could represent enzyme subunits or different states of enzyme aggregation.

20-Times greater loads of hemolysates from R. L., who had only 4% of normal HGPRT activity, displayed relatively flat profiles located between 60 and 80 mm along the gel, about 15% farther than the HGPRT-M enzyme. The dispersion of the radioactive zone of the HGPRT variant has suggested that the L$^-$ variant may also consist of several components. The profiles produced by over 100-times larger loads of hemolysate from J. R., a patient with the Lesch–Nyhan syndrome, generated only a very small amount of IMP and a mobility similar to that of the L$^-$ variant.

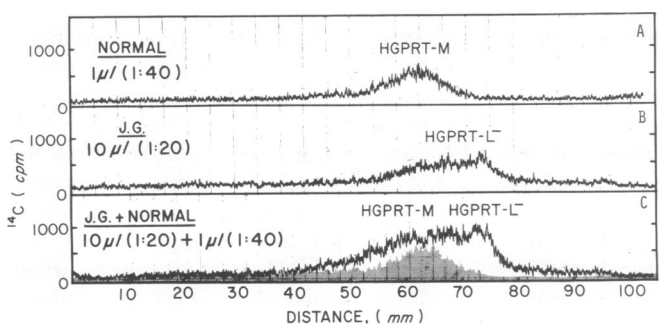

FIG. 2. Activation of partially deficient enzyme by normal hemolysate by polyacrylamide gel electrophoresis: (*A*) activity in 1 μl of 1:40 hemolysate from a normal individual; (*B*) activity in 10 μl of 1:20 hemolysate from a patient (J. G.) displaying 4% of normal activity in erythrocytes; and (*C*) activity in a mixture of 10 μl of 1:20 hemolysate from patient J. G. and 1 μl of 1:40 hemolysate from a normal individual. The *shaded area* in (*C*) is a copy of the profile shown in (*A*).

The profiles observed in hemolysate from S. F., half-sister of R. L., whose erythrocytes contained 34% of normal activity, differed from both the normal and variant patterns (Fig. 1). Her profiles consisted of two distinct zones of activity. This result suggested that she had two different HGPRT enzyme proteins, which would be consistent with heterozygosity. One enzyme migrated at the same rate as HGPRT-M, and the other at the rate of HGPRT-L$^-$ variant. However, the amount of activity in the L$^-$ area was considerably greater than in hemolysates of her brothers and cousins, in whom 100% of the cells contained the L$^-$ enzyme.

When the activity of erythrocytes of normal individuals is set at 100%, hemizygotes with the L$^-$ variant have 4%. S. F.'s red cells displayed 34% activity. A heterozygous cell population that would yield 34% activity would be expected to consist of about 31% cells with normal enzyme and 69% with the variant. In such a case the distribution of radioactive IMP in the electrophoretic profiles would approximate 91% in the HGPRT-M zone and 9% in the HGPRT-L$^-$. However, radioactivity found in the HGPRT-M zone represented 63% and that found in the L$^-$ zone, 37% of the total. The amounts of activity found in the zone of the variant enzyme could in no way be accounted for by any calculable ratio of the two cell populations.

Activation of partially deficient HGPRT variant by the normal hemolysate

An explanation for this unusual activity in the variant zone was sought through experiments in which hemolysates with the L$^-$ variant were mixed with hemolysates from normal individuals and subjected to electrophoretic separation. The results are shown in Fig. 2. The amount of activity present

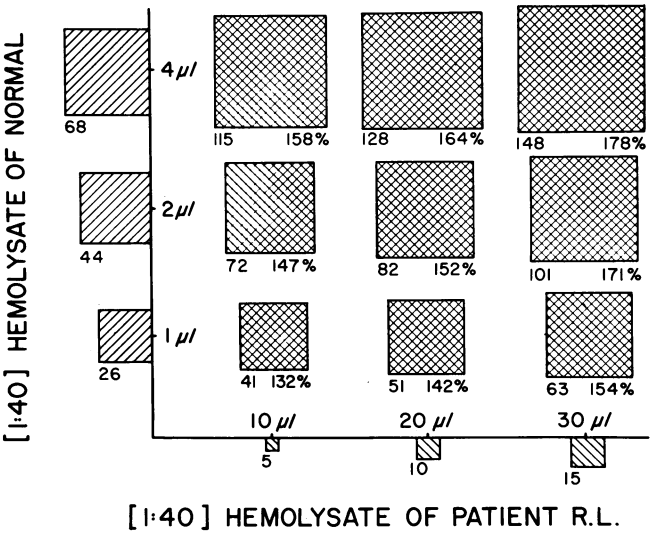

FIG. 3. Activation of HGPRT-L$^-$ variant by hemolysate from a normal individual with polyacrylamide gel electrophoresis. Aliquots of hemolysates from patient R. L. and from a normal individual and mixtures of the two were electrophoresed on gels, incubated in substrate solution containing 5 nmol (0.25 μCi) of [8-^{14}C]hypoxanthine per ml, fixed in $LaCl_3$ solutions washed in water, and screened for ^{14}C. *Numbers* on the *left* at the bottom of the squares represent nmol of [8-^{14}C]IMP, and on the *right*, the percent of [8-^{14}C]IMP recovered in the mixture, with 100% representing the sum of the normal and the patients' aliquots assayed alone.

in the profile of the mixed sample was considerably greater than the sum of the areas of the profiles of the two separate samples. Furthermore, superimposition of the normal profile (shaded area) on the profile of the mixed sample demonstrated that the increase in radioactivity was predominantly in the region occupied by the L⁻ variant.

This activation of the HGPRT-L⁻ variant was then explored systematically. Different amounts of 1:40 hemolysates from individuals with normal enzyme and from patients with the HGPRT-L⁻ variant, as well as mixtures of the two, were electrophoresed on the gel (Fig. 3). As indicated along the abscissa, 10-, 20-, and 30-μl aliquots of 1:40 hemolysate from a patient with 4% of normal activity generated 5, 10, and 15 nmol IMP. In the same experiment, as shown on the ordinate, 1-, 2-, and 4-μl aliquots of 1:40 hemolysate from a normal individual generated 26, 44, and 68 nmol IMP. The activity generated by mixtures of these aliquots of the two hemolysates (*cross-hatched squares*) was notably greater than the sum of the activities applied. In some instances the activity recovered was almost doubled. The tracings from which Fig. 3 was calculated indicate that large loads of protein do not alter the location of the enzyme.

Activation of the totally deficient variant by normal enzyme

More dramatic evidence for the activation of deficient enzyme by the normal one was obtained with hemolysates of patients with the Lesch–Nyhan syndrome (Fig. 4). The amount of activity in the area of the LN variant is much greater in the profile produced by the mixed sample than in the one produced by the LN variant alone. The activity in the area of normal enzyme (*shaded area*) was essentially unchanged in the mixture.

Activation of deficient enzyme by filtration through Sephadex G-25

When hemolysates from individuals with normal activity and from patients were mixed and assayed for HGPRT activity without electrophoresis, the amount of activity recovered was always equal to the sum of the two samples added (Fig. 5). However, electrophoresis on gel was not the only condition for activation. When the same mixtures were filtered

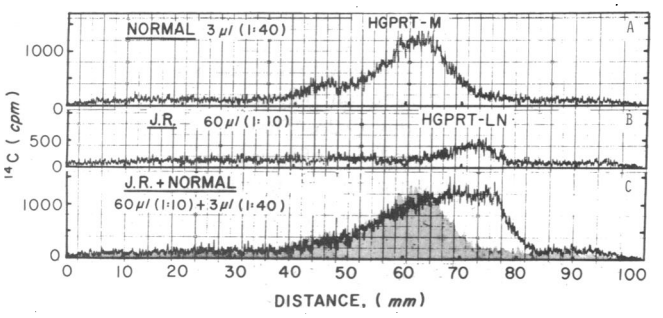

FIG. 4. Activation of HGPRT-LN variant by hemolysate from a normal individual with gel electrophoresis: (*A*) activity produced by 3 μl of 1:40 hemolysate from an individual with normal activity, (*B*) activity produced by 60 μl of 1:10 hemolysate from a patient (J. R.) with the Lesch–Nyhan syndrome, and (*C*) activity produced by a mixture of 60 μl of 1:10 hemolysate from patient J. R. and 3 μl of 1:40 hemolysate from an individual with normal activity. The *shaded area* in profile (*C*) is a copy of that in (*A*). These gels were developed in substrate solution containing 10 nmol (0.5 μCi) of [8-¹⁴C]hypoxanthine per ml.

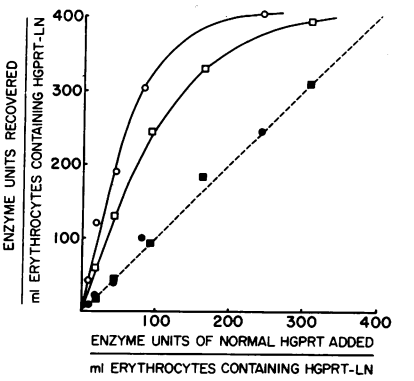

FIG. 5. Activation of HGPRT-LN variant by purified normal enzyme and by hemolysate from a normal individual by Sephadex G-25 filtration: hemolysate from a Lesch–Nyhan patient containing hemolysate from a normal individual before (■) and after (□) filtration, and hemolysate from a Lesch–Nyhan patient containing purified normal enzyme before (●) and after (○) filtration.

together through a Sephadex G-25 column, they emerged together in the breakthrough component, and the amount of activity recovered in some instances rose consistently to 600% (Fig. 5). Activation of the LN variant could also be regularly produced by purified normal enzyme. The degree of activation by purified enzyme was always greater than by hemolysate. Similarly, the purified enzyme also activated the L⁻ variant.

In contrast, attempts to activate HGPRT-LN by addition of HGPRT-L⁻ or vice versa failed both in gel filtration and in electrophoresis. In these experiments the activity recovered always equalled no more than the sum of the activities added. Separate filtration of hemolysates from normal individuals and from patients with LN or L⁻ variants did not increase the activity of HGPRT. Furthermore, the activity of hemolysates separately filtered and then combined always equalled the activities added.

Assessment of activation with purified enzyme iodinated with ^{125}I

In order to elucidate the mechanism of activation, we used purified enzyme labeled with ^{125}I. Iodination had very little effect on the specific (enzymatic) activity of the enzyme. Aliquots representing as little as 0.7–9 μg of iodinated enzyme containing up to 15,000 dpm of ^{125}I were added to different aliquots of 1:10 hemolysate of a patient with the Lesch–Nyhan syndrome and electrophoresed on the gel. The gels were then assayed for distribution of ^{125}I activity and for their enzymatic activity (Fig. 6). The iodinated purified enzyme consisted of one small component and a large one in the HGPRT-M zone between 60 and 70 mm. The patterns obtained by measurment of enzyme activity and ^{125}I were essentially identical.

When iodinated enzyme was added to erythrocyte hemolysate of M.P., iodinated protein appeared in the zone of the HGPRT-LN variant, 15% ahead of HGPRT-M (Fig. 6*B*). No such change in profile occurred when purified iodinated enzyme was added to normal hemolysate. The transfer of the iodinated protein into the region of the HGPRT-LN variant was also manifested by a notable increase in enzymatic activity in this otherwise almost inactive zone. Since the amount of added purified enzyme was very small (0.7 μg), there is

no doubt that most of the radioactivity generated in the HGPRT assay with [8-^{14}C]hypoxanthine was produced by [8-^{14}C]IMP, and only very little by ^{125}I. With increase in the size of the hemolysate sample there was a progressive decrease in ^{125}I activity of the major peak and an increase in the activity of the faster-migrating minor component. This result appeared to indicate that the amount of normal enzyme used by the deficient enzyme was proportional to the size of the aliquot of the deficient enzyme. This reaction may be highly competitive.

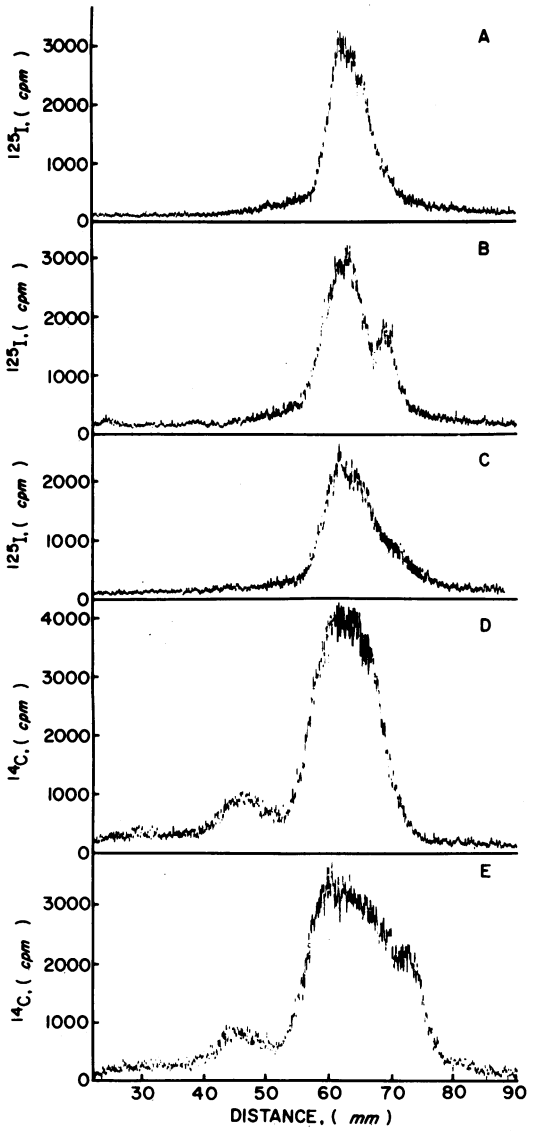

FIG. 6. Activation of HGPRT-LN variant by purified normal enzyme iodinated with ^{125}I: (*A*) distribution of ^{125}I in the profile produced by 40 μl (28 μg) of purified enzyme HGPRT No. 52, (*B*) distribution of ^{125}I in the profile of a mixture of 40 μl (28 μg) of [^{125}I]HGPRT No. 52 and 40 μl of 1:10 hemolysate from patient M. P. with the Lesch-Nyhan syndrome, (*C*) distribution of ^{125}I in the profile of a mixture of 40 μl (28 μg) of [^{125}I]HGPRT No. 52 and 40 μl of 1:10 hemolysate from a normal individual, (*D*) distribution of [8-^{14}C]IMP in the profile produced by 1 μl (0.7 μg) of [^{125}I]HGPRT No. 52, and (*E*) distribution of [8-^{14}C]-IMP in the profile produced by a mixture of 1 μl (0.7 μg) of [^{125}I]-HGPRT No. 52 and 40 μl of 1:10 hemolysate from a normal individual.

DISCUSSION

These studies indicate an unusual activation of two deficient variants of HGPRT by normal enzyme. Activation was demonstrated with the variant seen in patients with the Lesch-Nyhan syndrome and with the L$^-$ variant seen in patients with partial deficiency of HGPRT. It was demonstrable with purified enzyme and hemolysate.

The significance of these observations is heightened by the fact that the initial observation indicated that this process may go on *in vivo*. It was the unusual profile in which there were large zones of activity in both the normal and the variant area in a presumed heterozygote for partial deficiency that initiated this investigation. This patient was unusual because in heterozygotes for the Lesch-Nyhan syndrome there is hemizygous expression of only the normal phenotype in erythrocytes (9), presumably because of selective processes early in embryonic life. Measurements of total enzyme activity in a small number of obligate heterozygotes suggests that this is also true for the partial deficiencies. However, in this case it is clear that there are exceptions (10, 11). The proportion of these exceptions remains to be established. Furthermore, the occurrence of activation in the heterozygote indicates that this proportion can only be established by electrophoresis, for a total activity in the range of the normal could reflect the influence of the normal enzyme plus an activation of the variant in a hemolysate containing two cell populations.

Experiments in which the variant enzyme was activated with purified normal enzyme that was labeled with ^{125}I indicated that deficient enzymes incorporate components of normal enzyme into their structure. In this way the variant enzyme was brought to a form in which its catalytic function was performed more efficiently. At the same time, this transfer from normal enzyme to variant had very little effect on the electrophoretic properties of the variant. These observations suggest that the process under study is a protein-protein interaction. There may be an exchange of subunits.

We have assumed that the process under investigation is an activation of the variant enzyme by the normal enzyme. It is, of course, conceivable that the reverse is the case and the normal enzyme is being activated by the variant. This seems less likely because of the fact that the increase in activity is seen in the area of the variant protein on electrophoresis.

These interactions were observed to take place only during electrophoretic separations on polyacrylamide gel or gel filtration through Sephadex G-25. They have never been seen when mixtures of filtered or unfiltered deficient and normal enzymes were put together in the test tube. Physical actions of the gels needed to promote the protein-protein interaction could reflect a "stretching" of protein molecules by the gel matrix or an actual separation of subunits.

The observed activation of variant HGPRT after addition of hemolysate from a normal individual could obviously reflect the addition of small molecules, as well as the normal HGPRT enzyme. After all, several molecules—including Mg, sulfhydryl compounds, nucleotides, and nucleosides—have an effect on HGPRT activity. However, it does not seem likely that such small molecules are involved in the mechanism of the activation, because of the following observations. Activation has been achieved at dilutions as high as 1:600. Separate filtration, with or without subsequent

combination, of normal or variant HGPRT did not alter their activity. They must be filtered together. Most important, the activation has been clearly demonstrated with purified HGPRT protein.

There are precedents for protein–protein interactions leading to the activation of an inactive variant enzyme. In studies of *Escherichia coli lac*$^-$ mutants that had virtually no β-galactosidase activity, the mutant enzymes were activated up to 690-fold by the addition of specific antisera prepared in rabbits to the normal enzyme (16). Interaction with the antibody also stabilized the mutant enzyme against heat activation (17). This protein–protein interaction between antibody and enzyme was thought to produce a conformational change in the enzyme protein, leading to an opening up that made the active site available to substrate. It is possible that the activation of HGPRT variants could involve similar phenomena.

There are other instances in which a protein combines with an otherwise slightly active protein to form an active enzyme system with high specificity for a substrate. For example, lipoprotein lipase has very little hydrolytic activity of its own. However, when it combines with high-density lipoproteins, especially those that have glutamic acid or alanine carboxyterminal moieties, this lipoprotein lipase becomes highly active (18). The deficient β-glucosidase found in patients with Gaucher's disease is activated by what appears to be an acid glycoprotein of normal spleen (19).

Activation of HGPRT variants by normal enzyme has several potential practical implications. It provides a new approach to the study of molecular forms of deficient enzymes. It will be of interest to determine the generality of the method. In the second place, it could be helpful in the isolation of variant enzymes. Finally, evidence that an inactive enzyme can be activated could lead to the development of treatment. Especially if small molecules could be used that produced activation or restoration of biological activity of the enzyme, this principle could form the basis for effective therapy in HGPRT deficiency.

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